Archives of Current Medical Research
Arch Curr Med Res 2021;2(2):98-106

Received: 14 January 2021
Revised: 18 April 2021
Acccepted: 7 May 2021
DOI: 10.47482/acmr.2021.22

ORIGINAL ARTICLE

# Investigation of the Effect of Pentoxifylline and Tocopherol on Osseous Healing Following Tooth Extraction in Bisphosphonate-Administered Rats

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## Abstract

**Background:** The incidence of medication-related osteonecrosis of the jaw has increased with the widespread use of bisphosphonates. Present study aimed to evaluate the effect of pentoxifylline and/or tocopherol alone or in combination, on bisphosphonate-induced osteonecrosis in tooth extracted rat jaw.

**Methods:** 24 rats were randomly assigned to 4 groups and each animal received intraperitoneal zoledronic acid injection 0.06 mg/kg/week for 3 weeks. Following the zoledronic acid application, the lower right first molars of the rats were extracted on day 22. Starting from the day of tooth extraction animals received intraperitoneal pentoxifylline and/or tocopherol injections. Fourteen days later all rats were sacrificed. RANKL and osteoprotegerin (OPG) in blood were measured, and mandibles were examined histologically. When the inter-group differences were evaluated, the Kruskal Wallis-H Test and the Chi-square analysis were used.

**Results:** Each groups' serum RANKL, OPG and RANKL/OPG levels did not reveal any statistically significant differences. There were no statistically significant differences in terms of bone necrosis, abscess formation, inflammation, osteoblastic/osteoclastic activity, bone cellularity and epithelial integrity.

**Conclusions:** Pentoxifylline and/or tocopherol injections alone or in combination did not have any statistically significant effect on the jaw following tooth extraction in bisphosphonate-induced rat animal model.

**Key words:** Bisphosphonates-Associated Osteonecrosis of the Jaw, Pentoxifylline, Tocopherol.

***Cite this article as:*** *Tuncay E, Öztürk M, Tanıdır AN, Şengüven B, Paşaoğlu ÖT. Investigation of the effect of pentoxifylline and tocopherol on osseous healing following tooth extraction in bisphosphonate-administered rats. Arch Curr Med Res. 2021;2(2):98-106*

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## INTRODUCTION

Bisphosphonates are a class of drugs that have been widely used since the end of the 20th century in the treatment of systemic diseases such as osteoporosis, Paget's disease, multiple myeloma, cancers with bone metastases and hypercalcemia due to malignancy (1). The primary biological effect of all bisphosphonates occurs by inhibiting the bone resorption process through inhibitory activity on osteoclasts. Thus, bisphosphonates disrupt the mechanism of bone regeneration (2).

In the beginning, the side effects of these drugs were known to be acute phase responses, gastrointestinal and renal side effects. Another side effect of this drug was first reported by Marx in 2003, as osteonecrosis of the jaw (3). This condition was named "Medication-Related Osteonecrosis of the Jaw (MRONJ)" in 2014 according to the declaration issued by the Association of American Oral and Maxillofacial Surgeons (AAOMS) (2). AAOMS defines it as intraorally exposed bone, after treatment with anti-resorptive agents, persistent over a period of 8 weeks and no history of radiation therapy or metastases in the jaws (4).

The current treatment of MRONJ, reported by AAOMS, is based on eliminating or reducing the complaints of the patients. However, the expected results may not be obtained with this treatment protocol. Therefore, apart from the medical and/or surgical treatments of MRONJ, new methods and agents, which are thought to influence the bone and soft tissue healing in the positive direction, are being investigated (2, 5).

The aim of this study was to prove the preventive effect of pentoxifylline and tocopherol on bisphosphonate-related osteonecrosis following tooth extraction in bisphosphonate-administered rats.

## MATERIALS and METHODS

### Ethics Committee Approval and the Study Setting

This study was conducted in accordance with Gazi University Animal Experiments Local Ethics Committee Presidency Permission No. 66332047-604.01.02/139-17808, date: 05.08.2013.

The experimental phases of our study were carried out at the Gazi University Experimental Research Center and Animal Laboratory (GUERCAL), the biochemical evaluations were performed at GUERCAL, and the histopathological evaluations were performed at Gazi University Faculty of Dentistry, Department of Oral Pathology.

### The Characteristics of the Experimental Animals Used in the Study and the Optimized Criteria

In our study, 24 Wistar female healthy albino rats (200-250g) were used, which were obtained from GUERCAL. During the study, the care for the animals was provided at GUERCAL. Maintenance care for all animals was given in the same room by placing 6 rats in each cage. The rats were kept in a room with a 12-hour light and dark cycle, at 21-24°C, and 40-45% humidity. During the study, the rats were fed with standard dry pellet diet and water.

Zoledronic acid (ZA) 4 mg/5ml intravenous (IV) infusion (Novartis Pharma AG, Switzerland), which is one of the nitrogen-containing bisphosphonate group of drugs, was used to create animal models with MRONJ in the study.

### Surgical Protocol

All rats in the study received an intraperitoneal injection of ZA at 0.06 mg/kg per week for 3 weeks. On the 22nd day, mandibular right first molar tooth extraction was performed using the Bien Elevator (Medisporex CE 7811, Sialkot) and Deciduous Tooth Bayonet (Jensen JP-1 JDK111 CE, North Haven) in each of the subjects that had been prepared for surgery, by the same physician.

### Study Groups

Following the teeth extraction, 24 rats were randomly divided into four groups, with six rats in each group. The subgroups and the distribution of the type of injections and the doses after the extraction were as follows (Table 1):

**Table 1. Grouping of experimental animals**

| Group | Bisphosphonates application prior to tooth extraction | The applicable agent after tooth extraction | Rat numbers in the groups |
|---|---|---|---|
| Group A | Intraperitoneal Zoledronic Acid | Intraperitoneal Saline Solution | 6 |
| Group B | Intraperitoneal Zoledronic Acid | Intraperitoneal Alpha-Tocopherol | 6 |
| Group C | Intraperitoneal Zoledronic Acid | Intraperitoneal Pentoxifylline | 6 |
| Group D | Intraperitoneal Zoledronic Acid | Intraperitoneal Pentoxifylline and Alpha-Tocopherol | 6 |

Group A (Control Group): The rats in this group received daily intraperitoneal injections of isotonic NaCl solution.

Group B (Tocopherol Group): The rats in this group received daily intraperitoneal injections of 20 mg/kg/day of tocopherol (300 mg intramuscular ampule; Aksu Pharmaceutical, Turkey) for 14 days.

Group C (Pentoxifylline Group): The rats in this group received daily intraperitoneal injections of 50 mg/kg/day of pentoxifylline (100 mg/5 ml IV ampule; Berksam Pharmaceutical Trade. Inc., Turkey) for 14 days.

Group D (Tocopherol and Pentoxifylline Group): The rats in this group received daily intraperitoneal injections of 50 mg/kg/day of pentoxifylline and 20 mg/kg/day of tocopherol for 14 days.

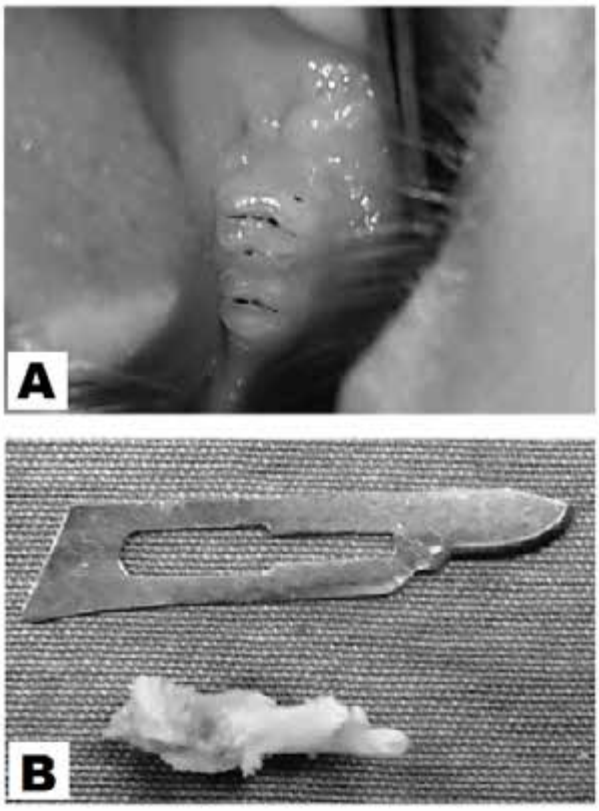
### Blood Sample Collection and Sacrification

The euthanasia procedure was performed on the 36th day when the experiment had been completed.

The blood samples were evaluated biochemically. A 3 ml sample of blood was collected in a sterile glass tube with clot activator, and without waiting, it was centrifuged at 3000 rpm for 10 minutes (Selecta Centronic-BL, J.P. SELECTA S.A., Barcelona). After centrifugation, the plasma portion was moved into 1.5 ml eppendorf tube to be evaluated with the ELISA kit (Rat OPG-RANKL Eliza Kit, Elabscience Biotechnology Co. Ltd., Japan).

After euthanasia, mandibular bone blocks where the tooth extraction was performed were removed for the extraction sockets to be examined histopathologically. The obtained bone blocks were kept in biopsy containers filled with 10% formaldehyde (Figure 1).

**Figure 1. Fourteen day after the tooth extraction images of the intraoral space (A) and resected hemimandible (B) from that space are shown**




### Biochemical Method

Cell-free plasma was obtained separately from all subjects. The parameters were studied as suggested by the manufacturer with ELISA kits (Rat OPG-RANKL Eliza Kit, Elabscience Biotechnology Co. Ltd., Japan).

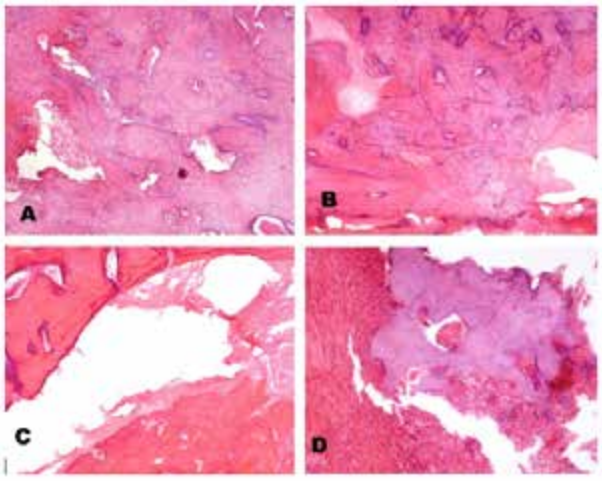
### Histopathological Method

The samples obtained after sacrification were fixed for 24-72 hours in 10% buffered formalin solution. Subsequently,

decalcification in 10% formic acid was provided. Sagittal plane section samples of 4-5 $\mu$m thickness were obtained from the tissues for routine hematoxylin-eosin staining to the adhesive slides (Surgipath, X-tra Adhesive Microslides, Illinois, USA). The sections were evaluated under the light microscope Leica DM 4000 B (Leica Microsystems GmbH. Wetzlar, Germany).

Presence of necrotic bone, bone cellularity, osteoblastic/osteoclastic activity, microorganism presence, presence of abscess and the density of inflammatory cell infiltration were evaluated in the hematoxylin-eosin stained sections from the mandibular bone blocks (Figure 2).

**Figure 2. Histological sections of extracted sockets are shown for each group with each groups letter (Hematoxylin-eosin, x200)**




The inflammatory cells were counted in the defect (also healing) field in the hematoxylin-eosin sections. The inflammation density was scored with a four-graded system reported by Hirshberg et al (6).

### Statistical Analysis

The data obtained in this study were analyzed using the SPSS 20 software package.

The Shapiro Wilk's test was used in the case of normal distribution of the variables, due to the number of units. The significance level was accepted as 0.05 when the results were interpreted (When $p<0.05$, the variables were considered not to have come from normal distribution, and when $p>0.05$, the variables were considered to have come from normal distribution).

When the inter-group differences were evaluated, the Kruskal Wallis-H Test was used in the case of variables not coming from normal distribution. The Chi-square analysis was used to evaluate the relationship of the nominal variables between the groups. The results were interpreted as 0.05 being accepted as the significance level.

## RESULTS

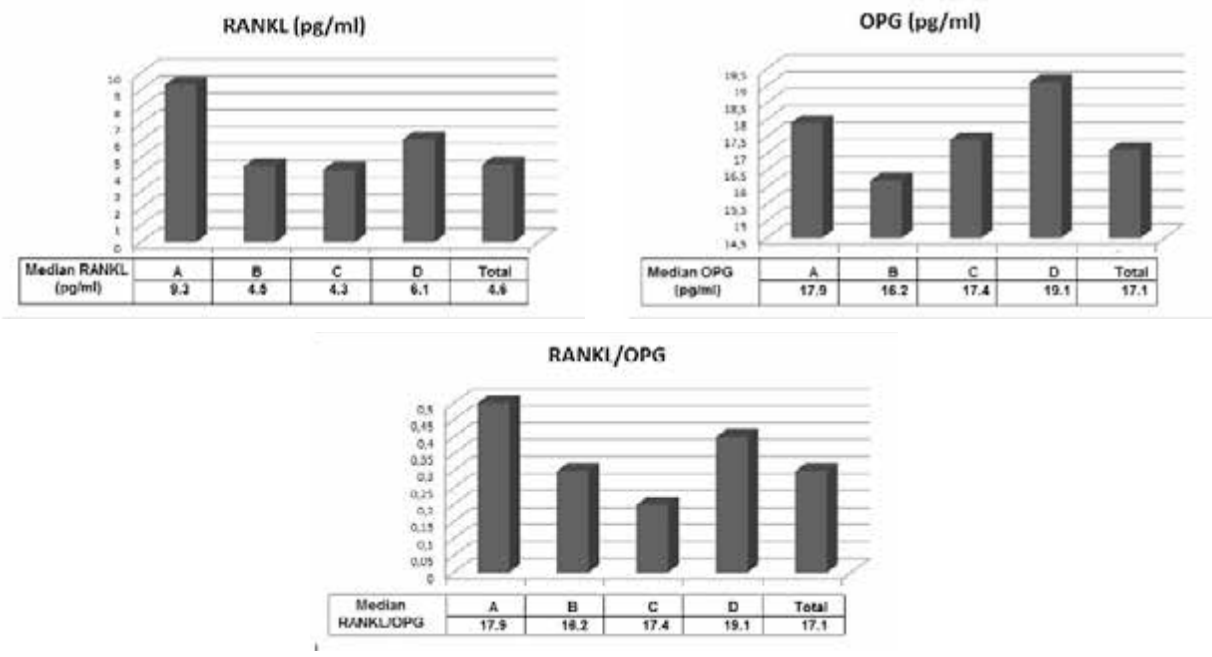
### Biochemical Results

The blood samples from the control and experimental groups were analysed using an ELISA kit; and RANKL, OPG and RANKL/OPG values were measured. (Table 2, Figure 3).

**Table 2. Absolute RANKL, OPG and RANKL/OPG serum levels of each rat in each group is provided with in the table**

| Group | Animal Number | RANKL (pg/ml) | OPG (pg/ml) | RANKL/OPG |
|---|---|---|---|---|
| Group A | 1 | 9.61 | 19.76 | 0.49 |
| Group A | 2 | 11.01 | 18.57 | 0.59 |
| Group A | 3 | 3.74 | 27.14 | 0.14 |
| Group A | 4 | 8.66 | 16.67 | 0.52 |
| Group A | 5 | 10.00 | 14.29 | 0.7 |
| Group A | 6 | 9.05 | 17.14 | 0.53 |
| Group B | 7 | 6.26 | 16.67 | 0.38 |
| Group B | 8 | 5.70 | 15.24 | 0.37 |
| Group B | 9 | 4.64 | 24.76 | 0.19 |
| Group B | 10 | 3.52 | 19.05 | 0.18 |
| Group B | 11 | 3.91 | 15.48 | 0.25 |
| Group B | 12 | 4.36 | 15.71 | 0.28 |
| Group C | 13 | 5.81 | 55.48 | 0.1 |
| Group C | 14 | 4.36 | 18.33 | 0.24 |
| Group C | 15 | 4.25 | 17.14 | 0.25 |
| Group C | 16 | 4.36 | 16.43 | 0.27 |
| Group C | 17 | 3.74 | 17.62 | 0.21 |
| Group C | 18 | 3.63 | 15.24 | 0.24 |
| Group D | 19 | 14.62 | 15.48 | 0.96 |
| Group D | 20 | 7.71 | 16.43 | 0.47 |
| Group D | 21 | 3.58 | 15.24 | 0.23 |
| Group D | 22 | 13.85 | 25.24 | 0.55 |
| Group D | 23 | 4.53 | 21.67 | 0.21 |
| Group D | 24 | 4.30 | 22.14 | 0.19 |

**Figure 3. The distribution graph of RANKL (pg/ml), OPG (pg/ml) and RANKL/OPG values according to the groups, respectively (Median value)**




## Histological Results

In all rats, in order to assess the healing process of the tooth extraction socket on the 14th day, eight variables such as necrotic bone presence, necrotic bone/total bone ratio, presence of abscess, level of inflammation, epithelial integrity, bone cellularity, and the presence or absence of osteoclastic and osteoblastic activity were evaluated. (Table 3).

**Table 3. The distribution of various histomorphological findings are provided with number (n) and percentage (%)**

| | | Group A | | Group B | | Group C | | Group D | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | N | % | n | % | n | % |
| Bone Necrosis | Absent | 2 | 33.3 | 0 | 0 | 2 | 33.3 | 2 | 33.3 | 6 | 25 |
| | Exist | 4 | 66.7 | 6 | 100 | 4 | 66.7 | 4 | 66.7 | 18 | 75 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Abscess Formation | Absent | 2 | 33.3 | 2 | 33.3 | 5 | 83.3 | 5 | 83.3 | 14 | 58.3 |
| | Exist | 4 | 66.7 | 4 | 66.7 | 1 | 16.7 | 1 | 16.7 | 10 | 41.7 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Inflammatory cells per field | Absent | 0 | 0 | 0 | 0 | 1 | 16.7 | 0 | 0 | 1 | 4.2 |
| | <15 | 2 | 33.3 | 1 | 16.7 | 4 | 66.7 | 4 | 66.7 | 11 | 45.8 |
| | 15-50 | 3 | 50 | 5 | 83.3 | 1 | 16.7 | 2 | 33.3 | 11 | 45.8 |
| | 50-75 | 1 | 16.7 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4.2 |
| | >75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Osteoblastic Activity | Absent | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Exist | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Osteoclastic activity | Absent | 6 | 100 | 6 | 100 | 6 | 100 | 5 | 83.3 | 23 | 95.8 |
| | Exist | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 16.7 | 1 | 4.2 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Bone Cellularity | Decreased | 0 | 0 | 0 | 0 | 1 | 16.7 | 0 | 0 | 1 | 4.2 |
| | Normal | 2 | 33.3 | 5 | 83.3 | 3 | 50 | 6 | 100 | 16 | 66.7 |
| | Increased | 4 | 66.7 | 1 | 16.7 | 2 | 33.3 | 0 | 0 | 7 | 29.2 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |
| Epithelial Integrity | Absent | 5 | 83.3 | 5 | 83.3 | 6 | 100 | 5 | 83.3 | 21 | 87.5 |
| | Exist | 1 | 16.7 | 1 | 16.7 | 0 | 0 | 1 | 16.7 | 3 | 12.5 |
| | Total | 6 | 100 | 6 | 100 | 6 | 100 | 6 | 100 | 24 | 100 |

In the defect field, hypercellular woven-trabecular new bone trabeculae accompanied by connective tissue composed of cell-rich active-swollen fibroblasts anastomosing with each other, with prominent osteoblastic columns were observed. Osteoclastic activity was observed in only one sample (Table 3).

Necrotic bone trabeculae were often observed in the superficial part of the defect field. It was observed that, generally, these constituted a very small part of the defect field.

Inflammation was not prominent in most of the samples. Only a few samples revealed intense abscess formation with acute inflammatory cell infiltration rich in neutrophils (Table 3). In general, mononuclear inflammatory cell infiltration, which can be considered within the normal range, was noticed in the samples.

No evidence of foreign body reaction was observed in the samples.

No significant difference was observed between the groups in terms of the amount of bone filling/bone quality, intensity of the inflammation or necrotic bone density. In one single rat (D6), a large area of bone necrosis between the trabeculae was observed. This image resembled a real bisphosphonate-related osteonecrosis.

### Statistical Results

No statistically significant difference was determined in the histopathological ( necrotic bone presence, bone cellularity, osteoclastic-osteoblastic activity, inflammation, abscess presence and necrotic bone/total bone percentage) and biochemical evaluation (RANKL, OPG, RANKL/OPG values).

## DISCUSSION

In recent years, an increase in the number of patients diagnosed with MRONJ, which is related to the use of bisphosphonates has been observed in the public. The presence of MRONJ significantly affects the patient's quality of life. However, there is no certain standard therapy for MRONJ or a specific protocol to reduce the development of MRONJ in the literature (7). Hence, the pathophysiology and the treatment of MRONJ should be clearly understood (8). Many studies have been conducted for the treatment of MRONJ in recent years (9){, #3}. In our study, we aimed to evaluate whether substances, we believe to reduce the risk of MRONJ, have any effect on bone healing in an experimental MRONJ model histopathologically and biochemically. To date, there are a few controlled studies conducted in this field with this subject.

In many experimental animal studies with experimental MRONJ models, rats were generally the preferred animals. ZA is one of the most potent amino bisphosphonates used intravenously, which is frequently associated with development of osteonecrosis. Therefore, the risk of MRONJ is higher in patients using ZA and it is one of the most commonly used bisphosphonates in experimental MRONJ studies (8, 10). In various studies using MRONJ models, ZA was administered to rats in doses of 0.25mg/kg subcutaneously, 0.04mg/kg IV and 0.02 mg/kg IV (11, 12). Since there was no precise dose protocol for ZA in rats and it would be inappropriate to apply higher doses of ZA to animal subjects compared to humans, because the drug has a cumulative effect. ZA application protocol for human patients is 4 mg/mL of ZA (IV) per month for a 70 kg person. Dose adjustments for rats were made according to human doses and it was decided that the drug be administered intraperitoneally instead of IV.

In many studies conducted to form an experimental MRONJ model, to increase the possibility of MRONJ formation, tooth extraction is the preferred surgical procedure. In Marx et al.'s study, it was reported that in 119 MRONJ patients, 68% was in the mandible, 28% in the maxilla and 4% in both jaws (13). In this study, because the study conditions were suitable and in parallel to the above-mentioned studies, right mandibular 1st molar tooth of all rats were extracted to increase the rate of MRONJ formation.

In Takami et al.'s study, it was reported that phosphodiesterase inhibitors such as pentoxifylline prevent the intracellular cAMP breakdown. They induce osteoclast formation in the mouse bone marrow, stimulate calvarial osteoblasts and trigger the secretion of RANKL and OPG cytokines in calvarial osteoblasts (14). In a study by Horiuchi et al., where the effects of parathyroid hormone and pentoxifylline on new bone formation was assessed, it was found that pentoxifylline increases new bone formation by increasing the BMP-2 (15). In another study conducted by Aydın et al. with a fracture model in rats, it was reported that 50mg/kg/day of pentoxifylline accelerated fracture healing in the

early healing period (16). There are antioxidant defense mechanisms in cells and the extracellular fluid that try to defuse the cytotoxic oxygen radicals. In humans, one of the substances that help prevent the damage of oxidants on the cell membrane is tocopherol, which is a type of vitamin E that has antioxidant property (17). In a study by Turk et al., a fracture model was formed in rats and 20mg/kg/day tocopherol was administered intraperitoneally to the study group. The fracture healing in rats, which were sacrificed on the 60th day, was evaluated, and the healing of the fractures in the study group were observed to be significantly better. Therefore, tocopherol was reported to have benefits in the early and late bone healing processes in clinical cases (18). Today, pentoxifylline is used with tocopherol in the treatment of osteoradionecrosis. These two drugs show a potent antifibrotic effect together. Studies have reported that the combination of pentoxifylline and tocopherol significantly decreases the chronic damage caused by radiotherapy (19). Epstein et al. published a case series of jaw osteonecrosis in six patients, due to use of ZA, ibandronic acid and alendronate. To treat these patients, in addition to antimicrobial therapy, oral 400 mg/day pentoxifylline and 400 mg/day tocopherol were prescribed. At the end of a 10-month follow-up, it was reported that the exposed bone surfaces shrank by 76% and all of the patients' complaints decreased (19). In a case report published by Magremanne et al., a jaw necrosis due to bisphosphonate use at stage 3 in the left lower jaw was detected. In addition to antimicrobial treatment, the patient was prescribed 400 mg pentoxifylline and 500mg tocopherol twice a day. After about 12 months, it was reported that the patient's complaints of paresthesia and pain had disappeared and the mucosal healing was completed (20). Studies on osteoradionecrosis and treatment of MRONJ in the literature have demonstrated that pentoxifylline and/or tocopherol may be useful in the treatment of jaw necrosis due to drugs. Considering the data obtained in previous studies on the subject, the present study evaluated the preventive effect of pentoxifylline and/or tocopherol on MRONJ formation.

New proteins, named RANKL, RANK and OPG, which have been defined in recent years, were presented as a series of cytokines connected to the TNF family, which is responsible for bone formation (21). RANKL and OPG are the major regulators of the molecular mechanism in osteoclast development and function. Both parameters may be used as genetic, immunohistochemical and serum indicators in in-vivo and in-vitro studies related to bisphosphonates (22). In this study, serum RANKL, OPG and RANKL/OPG values were used for biochemical evaluation to assess the systemic effects of pentoxifylline and tocopherol on bone metabolism.

Sonis et al. reported in their study that at the end of the surgical procedure following subcutaneous 0.075 mg/kg ZA administration on rats, 60% of the rats developed jaw necrosis. Bi et al. found no jaw necrosis after tooth extraction following intraperitoneal 0.125 mg/kg ZA administration (11). Huja et al. administered 0.1 mg/kg ZA per week for 9 weeks and no surgical procedure was performed. Although the specified ZA dose was much higher than the dose used in humans, no osteonecrosis was observed in the mice in the experiment group (23). Recreo et al. performed upper jaw 3rd molar tooth extraction in the experiment group following 0.1 mg/kg ZA administration 3 times a week for 9 weeks intraperitoneally. Osteonecrosis occurred in the extraction field in the experiment group, while in the group without tooth extraction, no osteonecrosis was reported (11). Considering the previous studies, the ZA dose, duration of use, injection type, and the presence of surgical procedure were all observed to affect the risk of MRONJ formation. In some studies in which high dose of ZA was administered, no osteonecrosis formation was observed; whereas in our study and in the similar studies mentioned above, osteonecrosis was observed even with low doses.

Although statistically insignificant, the histological findings in the osteonecrosis models formed in previous studies revealed similar results with our study. The necrotic bone trabeculae with empty lacunae containing no osteocytes were mostly observed in areas close to the surface of the defect. In the pentoxifylline study groups, lower rates of abscess formation and inflammation were determined. Therefore, we believe that application of pentoxifylline reduces the formation of these findings and reduces the risk of osteonecrosis.

RANKL and OPG play a major role in regulating the maturation and differentiation of osteoclasts (24). Bisphosphonates particularly affect the osteoclasts; therefore, they affect the RANKL-RANK-OPG metabolism. Thus, clarification of the effect of RANKL and OPG release on MRONJ development and the definition of the agents that increase or decrease the release of these two factors are very important for MRONJ and for other studies about MRONJ treatment. Mercatali et al. assessed the RANK,

RANKL and OPG values of 49 follow-up cancer patients who used IV ZA for 12 months. At the end of 12 months, the mean RANKL value decreased by 22%, the mean OPG value increased by 96%, and the RANKL/OPG ratio decreased by 56%. ZA is believed to affect the RANKL, RANK and OPG release due to its osteoclastic activity reducing effect (25). In our study, no significant difference was found between the groups in terms of serum RANKL, OPG, RANKL/OPG values ($p>0.05$ Kruskal-Wallis H Test). Considering the inhibitory effect of bisphosphonate use on RANKL release, the lower RANKL values obtained in groups B, C and D compared to group A resulted in the opinion that pentoxifylline and/or tocopherol injection reduces RANKL expression or has no effect on its stimulation. The lower RANKL values in the study groups resulted in the opinion that osteoclast activation, differentiation and apoptosis are lower, and thus, bone resorption is higher in the control group. In addition, considering the increasing effect of bisphosphonate use on OPG release, it is believed that with the lower OPG values found in groups B and C compared to group D may be caused by pentoxifylline and/or tocopherol application, which may have decreased the effect of bisphosphonates on OPG values and risk of MRONJ formation. Bisphosphonate application is known to decrease the RANKL/OPG ratio. Thus, the RANKL/OPG ratios of group B, C and D being less than group A, led to the conclusion that pentoxifylline and/or tocopherol injection has no effect on increasing the total bone mass.

Although there are studies in recent years associated with the effects of bisphosphonate use on RANKL and OPG release, there are no studies evaluating the effects of agents or surgical procedures that may have possible benefits on MRONJ treatment and RANKL and OPG values. Therefore, our study is the first in the literature about this topic.

Given these results, even with the low dose of ZA applied to rats intraperitoneally, with the human ZA dose taken as reference, osteonecrosis fields histologically compatible with MRONJ were detected. Furthermore, although application of pentoxifylline and/or tocopherol in the model of MRONJ formation risk, compared with the control group, statistically made no difference at all, when we analyzed the histopathological and biochemical data we observed differences between the groups. In particular, pentoxifylline and/or tocopherol injections were observed to positively affect the OPG.

The limiting factors of this study were as follows: when the model was created for MRONJ formation in the study groups, only one bisphosphonate type and dose was selected, and only a given single dose option for pentoxifylline and/or tocopherol administration was used. All animals were sacrificed at the same time so the bone healing parameters in different time periods could not be evaluated. In order to further assess the information on preventive and/or therapeutic effects of pentoxifylline and/or tocopherol in MRONJ; more extensive experimental and clinical trials are needed with the mentioned limitations overcame. Also, new bone formation should be assessed with 3-dimensional radiographic methods, as well as histopathological and histomorphometric evaluation.

**Declarations**

This research was supported by Scientific Research Projects of Gazi University with project number 03/2014-02. There is no conflict of interest.

This study was conducted in accordance with Gazi University Animal Experiments Local Ethics Committee Presidency Permission No. 66332047-604.01.02/139-17808, date: 05.08.2013.

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